UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
CHEMICAL SAFETY
AND POLLUTION
PREVENTION

Revised April 30, 2014
April 10, 2014

MEMORANDUM

SUBJECT: Efficacy Review for Brace;
EPA Reg. No. 777-99;
DP Barcode: D417453

FROM: Karen M. Hill, Ph.D.
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TO: Jacqueline Hardy RM34/ Thomas Luminello
Regulatory Management Branch II
Antimicrobials Division (7510P)

APPLICANT: Reckitt Benckiser, Inc.
399 Interpace Parkway
Parsippany, NJ 07054

Formulation from the Label:

Active Ingredient(s):	% by wt.
Alkyl (50% C_{14}, 40% C_{12}, 10% C_{16}) dimethyl benzyl ammonium saccharinate.....	0.10%
Ethanol.....	58.00%
Inert Ingredients.....	41.90%
Total.....	100.00%

I. BACKGROUND:

The product, BRACE (EPA Reg. No. 777-99), is an EPA-approved disinfectant (bactericide, tuberculocide, fungicide, virucide), sanitizer, mildewcide, and deodorizer for use on hard, non-porous surfaces in household, institutional, commercial, food preparation, animal care, and hospital or medical environments. The product is also for use as a soft surface sanitizer. The applicant requested to amend the registration of this product to add disinfection on hard non-porous surfaces claims for supplementary microorganisms. The applicant provided confirmatory efficacy data to support the additional and revised microorganism claims. All of the studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Suite 110, in Eagan, MN 55121 except for the SARS Coronavirus study which was conducted at MicroBioTest, Division of Microbac Laboratories, Inc. 105 Carpenter Drive, Sterling, VA 20164.

The data package contained a letter from the applicant to the Agency dated December 10, 2013, EPA Form 8570-35 (Data Matrix), proposed amended product label (amended date December 10, 2013), transmittal document, and forty-six (46) efficacy studies (MRIDs 492486-01 thru -46) with Statements of No Data Confidentiality Claims for all studies embedded in each respective MRID.

II. USE DIRECTIONS:

The product is designed for disinfecting hard, non-porous, non-food contact surfaces, including: appliances, bathtubs, bed frames, bed springs, bidets, blinds, cabinets, cages, chairs, changing tables, clean-up carts, counters, cribs, cuspidors, desks, diaper pails, dish pails, doorknobs, drains, dressing carts, drinking fountains, examination tables, faucets, fixtures, floors, furniture, garbage cans, garbage pails, highchairs, kennels, lamps, laundry hampers, light switches, linen carts, litter boxes, mattress covers, mirrors, outdoor patio furniture, pens, recycling bins, remote controls, salad bar sneeze guards, showers, sinks, sports equipment, stretchers, tables, telephones, toilets, tools, toys, walls, wheelchairs, whirlpool interiors, and windows. The proposed label indicates that the product may be used on hard, non-porous surfaces, including: crystal, cultured marble, enamel, glass, glazed ceramic, glazed porcelain, glazed tile, laminated surfaces, linoleum, Marlite, metal (i.e., brass, chrome, copper, stainless steel, tin), Parquet, plastic, sealed granite, synthetic marble, and vinyl.

Directions on the proposed amended label provide the following information regarding use of the product as a disinfectant:

(Pre-clean surfaces prior to use.) Hold can (container) upright 6" to 8" from surface. Spray 3 to 4 seconds until covered with mist. ((Gross) (Heavy) soil must be removed prior to application.)

(Let stand for 3 minutes then allow to air dry.) (For (Norovirus) (and) (*Mycobacterium bovis* BCG (Quant tuberculosis)) Let stand for 10 minutes then allow to air dry)

III. AGENCY STANDARDS:

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments:
The effectiveness of disinfectants for use on hard surfaces in hospital or medical environments must be substantiated by data derived using the AOAC Use-Dilution Method (for water soluble powders and liquid products) or the AOAC Germicidal Spray Products Test (for spray products), or the AOAC Hard Surface Carrier Test. The tests require that sixty carriers must be tested with each of 3 samples, representing 3 different batches, one of which is at least 60 days old or all tested batches at or below the active ingredient(s) lower certified limit(s), against a mean log density of at least 6 for *Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (ATCC 15442). To support products labeled as "disinfectants", killing on 59 out of 60 carriers for germicidal spray testing is required to provide effectiveness at the 95% confidence level. To pass performance requirements when using AOAC Hard Surface Carrier Test, tests must result in killing in 58 out of each set of 60 carriers for *Staphylococcus aureus* ATCC 6538; 57 out of each set of 60 carriers for *Pseudomonas aeruginosa* ATCC 15442 within ten minutes. For AOAC Use-Dilution testing, testing for each lot should be conducted on a different day. Thus, a total of three tests for *S. aureus* and three tests for *P. aeruginosa* are necessary. Sixty carriers are required per test, without contamination in the subculture media. The performance standard for *S. aureus* is 0-3 positive carriers out of sixty. The performance standard for *P. aeruginosa* is 0-6 positive carriers out of sixty. To be deemed an effective product, the product must pass all tests for both microbes.

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments (Additional Bacteria):
Effectiveness of disinfectants against specific bacteria other than those named in the AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Method, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method, must be determined by either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products as Disinfectants Method. Ten carriers must be tested against each specific microorganism with each of 2 product samples, representing 2 different product lots. To support products labeled as "disinfectants" for specific bacteria (other than those bacteria named in the above test methods), killing of the specific microorganism on all carriers is required.

Batch replication for modified tests:
Where batch replication has already been performed and accepted for a product registration with unmodified tests by the recommended methods, additional testing at the same use concentration under modified conditions (e.g., different exposure period, presence of organic soil or hard water, porous surface carriers, etc.) may be conducted with reduced batch replications as followed: For basic efficacy claims (e.g., sterilants, disinfectants, sanitizers), two samples, representing two different batches, instead of three; For supplemental efficacy claims (e.g., fungicides, virucides, and tuberculocides, one sample instead of two.

Virucides:
The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray

Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level. If the product is intended to be represented as a one-step virucidal, an appropriate organic soil (i.e.- 5 percent blood serum) should be included with the viral inoculum.

Disinfectants for Use as Fungicides (Against Pathogenic Fungi):
Effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data derived from each of 2 samples representing 2 different batches using the AOAC Fungicidal Test. The test should be conducted at 5, 10, and 15 minute exposure times. Alternatively, the AOAC Use Dilution Method, modified to conform with appropriate elements in the AOAC Fungicidal Test, may be employed. If the product is intended for use as a spray, the AOAC Germicidal Spray Products Test must be employed. Performance requirements for this test: the highest dilution that kills all fungal spores is the minimum effective concentration. Ten carriers for each of two samples representing two different batches of the product should be evaluated against *Trichophyton mentagrophytes* (ATCC 9533). The inoculum employed should provide a concentration of $1 \times 10^4 - 1 \times 10^5$ conidia per carrier. For the AOAC International Fungicidal Activity of Disinfectants test, all fungal spores at 10 and 15 minutes should be killed to support a 10 minute exposure time. For the AOAC International Use-Dilution Methods, all fungal spores on all 10 carriers should be killed in ≤ten minutes.

Supplemental Claims:
An antimicrobial agent identified as a "one-step" disinfectant or as effective in the presence of organic soil must be tested for efficacy with an appropriate organic soil load, such as 5 percent serum.

IV. BRIEF DESCRIPTION OF THE DATA:

The product's Certificate of Analysis which incorporated each tested lot was provided. Testing of the active ingredients concentration was performed by Smithers Avanza at 790 Main Street Wareham, MA. 02571, and Reckitt Benckiser Analytical laboratory. The results of the testing are given below.**

Lot	Onyxide Active Ingredient Concentration	Ethanol Active Ingredient Concentration
2028-029	0.083%	54.38%
2028-030	0.082%	54.66%
2028-031	0.082%	54.73%
1971-095	0.080%	54.55%

1971-101	0.080%	54.44%
1836-132**	0.090%	54.88%
1836-133**	0.089%	55.15%

All of the tested lots are below the active ingredient lower certified limit that is listed on the product's Confidential Statement of Formula as 0.09% for Onyxide and 56.26% for Ethanol.

Note: Testing was performed on test substances Formula # 1178-172 and Formula # e0029-002H which are identical to the product Brace.

1. **MRID 492486-01, "AOAC Germicidal Spray Method," Test Organisms: *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella enterica* (ATCC 10708) and *Staphylococcus aureus* (ATCC 6538). For product Brace. Study conducted at ATS Labs by Joshua Luedtke. Study completion date – October 11, 2013. Project Number A15060.**

The study was conducted against *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella enterica* (ATCC 10708) and *Staphylococcus aureus* (ATCC 6538). Testing was conducted using three batches of test substance Brace, Batch 2028-029, Batch 2028-030 and Batch 2028-031. Testing was performed according to using ATS Laboratory Protocol No. SRC62040513.GS.23 (copy provided). The product was received as ready to use (RTU) aerosol spray. Initial broth cultures of the test organisms were prepared by inoculation of 10 µL aliquots from a thawed, vortex mixed stock cryovial to an initial 10 mL tube of Synthetic Broth growth medium and were incubated for 24±2 hours at 35-37°C. Following incubation, a 10µL aliquot of each culture was transferred to individual 20 x 150 mm Morton Closure tubes containing 10 mL of culture medium (Daily transfer #1). The final test culture was incubated for 48 – 54 hours at 35-37°C. On the day of use, the *Pseudomonas aeruginosa* culture pellicle was carefully aspirated by vacuum aspiration. Care was taken to avoid disrupting the pellicle. All test cultures were vortexed and allowed to stand for ≥ 10 minutes prior to removing the upper portion of the culture for use in testing. A 0.20 mL aliquot of fetal bovine serum was added to 3.80 mL of each prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) per product batch, each in a Petri dish matted with two pieces of filter, were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C and with 54.8% relative humidity. Carriers were used within 2 hours of drying. For each lot of test substance, test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at room temperature 20.92-24.37°C with 28.45-36.28% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessels were shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

2. **MRID 492486-02, "AOAC Germicidal Spray Method," Test Organism: *Acinetobacter baumannii*, multi drug resistant (ATCC 19606). For product Brace. Study conducted at ATS Labs by Gracia Schroeder. Study completion date – October 22, 2013. Project Number A15064.**

The study was conducted against *Acinetobacter baumannii*, multi drug resistant (ATCC 19606). Testing was conducted using two batches of test substance Brace, Batch 2028-030 and Batch 2028-031. Testing was performed according to ATS Laboratory Protocol No. SRC62040513.GS.1 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loop of stock slant culture was transferred to an initial 10 mL tube of Nutrient Broth growth medium, mixed and incubated for 24±2 hours at 35-37°C. As the final test culture, a 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium and was incubated for 48-54 hours at 35-37°C. Following incubation, all test cultures were vortexed and allowed to stand for ≥ 10 minutes prior to removing the upper portion of the culture for use in testing. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C and at 55.8% relative humidity. Carriers were used within 2 hours of drying. For each lot of test substance, test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 25.58°C with 31.34% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessels were shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Antibiotic sensitivity testing was performed using a representative culture from the day of testing to verify the stated antibiotic resistance pattern. This testing was performed at the University of Minnesota Physicians Outreach Laboratory in Minneapolis, Minnesota and confirmed antibiotic resistance to many antibiotics. This testing was not performed under EPA GLP (40 CFR Part 160).

3. **MRID 492486-03, "AOAC Germicidal Spray Method," Test Organism: *Acinetobacter calcoaceticus*, (ATCC 17902). For product Brace. Study conducted at ATS Labs by Matthew Sathe. Study completion date – October 21, 2013. Project Number A15061.**

The study was conducted against *Acinetobacter calcoaceticus* (ATCC 17902). Testing was conducted using two batches of test substance Brace, Batch 2028-030 and Batch 2028-031. Testing was performed according to ATS Laboratory Protocol No. SRC62050613.GS (copy provided). The product was received as ready to use (RTU) aerosol spray. A loop of stock slant culture was transferred to an initial 10 mL tube of Nutrient Broth growth medium, mixed and incubated for 24±2 hours at 35-37°C. A 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10

mL of culture medium (daily transfer #1). The test culture was incubated for 48-54 hours at 35-37°C and was mixed thoroughly prior to use. An aliquot of fetal bovine serum was added to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C with 52.2% relative humidity. Carriers were used within 2 hours of drying. For each lot of test substance, test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 23.98°C with 31.60% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessels were shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: No protocol deviations occurred during this study.

4. **MRID 492486-04, "AOAC Germicidal Spray Method," Test Organism: *Bordetella pertussis*, (ATCC 12743). For product Brace. Study conducted at ATS Labs by Gracia Schroeder. Study completion date – October 23, 2013. Project Number A15108.**

The study was conducted against *Bordetella pertussis* (ATCC 12743). Testing was conducted using two batches of test substance Brace, Batch 2028-030 and Batch 2028-031. Testing was performed according to ATS Laboratory Protocol No. SRC62040513.GS.3 (copy provided). The product was received as ready to use (RTU) aerosol spray. From stock, Bordet Gengou Agar plates were inoculated with the test organism and incubated for 5 days at 35-37°C. Following incubation, the organism was suspended in Butterfield's buffer to approximately match 4.0 McFarland turbidity standards. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 25-30°C with 65% relative humidity. Carriers were used within 2 hours of drying. For each lot of test substance, test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 23.68°C with 43.21% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. Each test vessel was shaken thoroughly and vortex mixed. The entire volume of the subculture broths were individually transferred to the surface of a filter membrane (0.2 µm porosity), pre-wetted with 10.0 mL of sterile saline and filtered using a vacuum pump. Each filter membrane was washed with ≥50 mL of sterile saline and was then aseptically placed on the surface of a Bordet Gengou Agar plate. All plates

were incubated for 6 days at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: No protocol deviation or amendments were required for this study.

5. **MRID 492486-05, "AOAC Germicidal Spray Method," Test Organism: *Burkholderia cepacia*, (ATCC 17902). For product Brace. Study conducted at ATS Labs by Joshua Luedtke. Study completion date – October 21, 2013. Project Number A15103.**

The study was conducted against *Burkholderia cepacia* (ATCC 25416). Testing was conducted using one batch of test substance Brace, Batch 2028-030. Testing was performed according to ATS Laboratory Protocol No. SRC62040513.GS.4 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of Nutrient Broth growth medium, mixed and incubated for 24±2 hours at 35-37°C. A 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). One additional daily transfer was prepared. The final test culture was incubated for 48-54 hours at 35-37°C and was mixed thoroughly prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C with 40% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 23.37°C with 43.68% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessels were shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: No protocol deviation or amendments were required for this study.

6. **MRID 492486-06, "AOAC Germicidal Spray Method," Test Organism: *Corynebacterium diphtheriae*, (ATCC 11913). For product Brace. Study conducted at ATS Labs by Joshua Luedtke. Study completion date – October 15, 2013. Project Number A15104.**

The study was conducted against *Corynebacterium diphtheria* (ATCC 11913). Testing was conducted using one batch of test substance Brace, Batch 2028-030. Testing was performed according to ATS Laboratory Protocol No. SRC62040513.GS.6 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loop of stock slant culture was transferred to an initial 10 mL tube of Tryptic Soy Broth growth medium, mixed and incubated for 24±2 hours at 35-37°C. A 10 µL aliquot of this culture

was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). One additional daily transfer was prepared. The final test culture was incubated for 48-54 hours at 35-37°C and was mixed thoroughly prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. 10 individual glass slide carriers (18 mm x 36 mm) were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C with 40% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 23.39°C with 44.89% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Tryptic Soy Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessels were shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: No protocol deviation or amendments were required for this study.

7. **MRID 492486-07, "AOAC Germicidal Spray Method," Test Organism: *Enterobacter aerogenes,* multi drug resistant (ATCC 29571). For product Brace. Study conducted at ATS Labs by Joshua Luedtke. Study completion date – October 21, 2013. Project Number A15105.**

The study was conducted against *Enterobacter aerogenes,* multi drug resistant (ATCC 29751). Testing was conducted using one batch of test substance Brace, Batch 2028-030). Testing was performed according to ATS Laboratory Protocol No. SRC62040513.GS.7 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of Tryptic Soy Broth growth medium, mixed and incubated for 24±2 hours at 35-37°C. A 10 µL aliquot of this culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). One additional daily transfer was prepared. The test culture was vortexed and allowed to stand for ≥10 minutes prior to removing the upper portion leaving behind any clumps or debris and pooling the cultures in a sterile vessel for use in testing. The final test culture was incubated for 48-54 hours at 35-37°C and was mixed thoroughly prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C and at 40% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 23.60°C with 40.49% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessels

were shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Antibiotic sensitivity testing was performed using a representative culture from the day of testing to verify the stated antibiotic resistance pattern. This testing was performed at the University of Minnesota Physicians Outreach Laboratory in Minneapolis, Minnesota and confirmed antibiotic resistance. This testing was not performed under EPA Good Laboratory Practices (40 CFR Part 160).

Note: No protocol deviations occurred during this study.

8. **MRID 492486-08, "AOAC Germicidal Spray Method," Test Organism: *Enterococcus faecalis*, (ATCC 828). For product Brace. Study conducted at ATS Labs by Joshua Luedtke. Study completion date – October 3, 2013. Project Number A15106.**

The study was conducted against *Enterococcus faecalis* (ATCC 828). Testing was conducted using one batch of test substance Brace, Batch 2028-030. Testing was performed according to ATS Laboratory Protocol No. SRC62040513.GS.8 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of Thioglycolate Broth growth medium, mixed and incubated for 24±2 hours at 35-37°C. A 10 µL aliquot of this culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). One additional daily transfer was prepared. The final test culture was incubated for 48-54 hours at 35-37°C and was mixed thoroughly prior to use. The test culture was vortexed and allowed to stand for ≥10 minutes prior to removing the upper portion leaving behind any clumps or debris and pooling the cultures in a sterile vessel for use in testing. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 25-30°C with 65% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 23.39°C with 41.30% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessels were shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: No protocol deviation or amendments were required for this study.

9. **MRID 492486-09, "AOAC Germicidal Spray Method," Test Organism: Vancomycin Resistant *Enterococcus faecalis* - VRE (ATCC 51575). For product Brace. Study conducted at ATS Labs by Joshua Luedtke. Study**

completion date – October 7, 2013. Project Number A15107.

The study was conducted against Vancomycin Resistant *Enterococcus faecalis* - VRE (ATCC 51575). Testing was conducted using one batch of test substance Brace, Batch 2028-030. Testing was performed according to ATS Laboratory Protocol No. SRC62040513.GS.9 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of Thioglycolate Broth growth medium, mixed and incubated for 24±2 hours at 35-37°C. A 10 µL aliquot of this culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). One additional daily transfer was prepared. The final test culture was incubated for 48-54 hours at 35-37°C and was mixed thoroughly prior to use. The test culture was vortexed and allowed to stand for ≥10 minutes prior to removing the upper portion leaving behind any clumps or debris and pooling the cultures in a sterile vessel for use in testing. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 31 minutes at 25-30°C with 65% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 23.85°C with 42.08% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessels were shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Antimicrobial susceptibility testing was performed by ATS Labs for Vancomycin Resistant *Enterococcus faecalis* (ATCC 51575) to verify the antibiotic resistance pattern stated. The Kirby Bauer susceptibility assay was performed utilizing a representative culture from the day of testing and using vancomycin antibiotic disks to confirm resistance. In addition, antibiotic sensitivity testing was performed at the University of Minnesota Physicians Outreach Laboratory in Minneapolis, MN.

Note: No protocol deviation or amendments were required for this study.

10. **MRID 492486-10, "AOAC Germicidal Spray Method," Test Organism: *Enterococcus faecium,* multi drug resistant (ATCC 51559). For product Brace. Study conducted at ATS Labs by Matthew Sathe. Study completion date – October 21, 2013. Project Number A15059.**

The study was conducted against *Enterococcus faecium,* multi drug resistant (ATCC 51559). Testing was conducted using two batches of test substance Brace, Batch 2028-030 and Batch 2028-031. Testing was performed according to ATS Laboratory Protocol No. SRC62040513.GS.10 (copy provided). The product was received as ready to use (RTU) aerosol spray. From stock, sufficient Tryptic Soy agar plates with 5% sheep blood (BAP) were inoculated with the test organism. The plates

were incubated for four days at 35-37°C aerobically. Following incubation, the organism was suspended in sterile diluent to match a 0.5 McFarland turbidity standard. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 25-30°C with 62% relative humidity. The carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 25.0°C with 28.1% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessels were shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Antibiotic sensitivity testing was performed using a representative culture from the day of testing to verify the stated antibiotic resistance pattern. This testing was performed at the University of Minnesota Physicians Outreach Laboratory in Minneapolis, Minnesota and confirmed resistance. This testing was not performed under EPA Good Laboratory Practices (40 CFR Part 160).

Note: No protocol deviations occurred during this study.

11. MRID 492486-11, "AOAC Germicidal Spray Method," Test Organism: *Escherichia coli* O157:H7 (ATCC 43888). For product Brace. Study conducted at ATS Labs by Jill Ruhme. Study completion date – October 16, 2013. Project Number A15091.

The study was conducted against *Escherichia coli* O157:H7 (ATCC 43888). Testing was conducted using one batch of test substance Brace, Batch 2028-030. Testing was performed according to ATS Laboratory Protocol No. SRC62040513.GS.11 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of Synthetic Broth growth medium, mixed and incubated for 24±2 hours at 35-37°C. A 10 µL aliquot of this culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C and was mixed thoroughly prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C with 50% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet

for 2 minutes at 24.8°C with 38.8% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessels were shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: No protocol deviations occurred during this study.

12. **MRID 492486-12, "AOAC Germicidal Spray Method," Test Organism: Extended-Spectrum Beta-lactamase (ESBL) producing *E. coli* (ATCC BAA-196). For product Brace. Study conducted at ATS Labs by Jill Ruhme. Study completion date – October 17, 2013. Project Number A15092.**

The study was conducted against Extended-Spectrum Beta-lactamase (ESBL) producing *E. coli* (ATCC BAA-196). Testing was conducted using one batch of test substance Brace, Batch 2028-030. Testing was performed according to ATS Laboratory Protocol No. SRC62040513.GS.12 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of Synthetic Broth growth medium, mixed and incubated for 24±2 hours at 35-37°C. A 10 µL aliquot of this culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The test culture was vortexed and allowed to stand for ≥10 minutes prior to removing the upper portion leaving behind any clumps or debris and pooling the cultures in a sterile vessel for use in testing. The final test culture was incubated for 48-54 hours at 35-37°C and was mixed thoroughly prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C with 41% relative humidity. The carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 24.6°C with 39.7% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessels were shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Antibiotic susceptibility testing of Extended Spectrum Beta-lactamase (ESBL) *Escherichia coli* (ATCC BAA-196) was performed by ATS Labs to verify the antimicrobial resistance pattern of an ESBL. The Etest® assay was performed on a representative culture from the day of testing to confirm that the test organism is antibiotic resistant. In addition, antibiotic sensitivity testing was performed at the University of Minnesota Physicians Outreach Laboratory in Minneapolis, Minnesota and confirmed resistance.

This testing was not performed under EPA Good Laboratory Practices (40 CFR Part 160).

Note: No protocol deviations or amendments were required for this study.

13. MRID 492486-13, "AOAC Germicidal Spray Method," Test Organism: *Haemophilus influenzae*, (ATCC 33930). For product Brace. Study conducted at ATS Labs by Gracia Schroeder. Study completion date – October 15, 2013. Project Number A15109.

The study was conducted against *Haemophilus influenza* (ATCC 33930). Testing was conducted using two batches of test substance Brace, Batch 2028-030 and Batch 2028-031. Testing was performed according to ATS Laboratory Protocol No. SRC62040513.GS.13 (copy provided). The product was received as ready to use (RTU) aerosol spray. From the stock, Chocolate agar plates were inoculated with the test organism and incubated for 4 days at 35-37°C in CO_2. Following incubation, the culture was suspended in Butterfield's buffer to approximately match a 4.0 McFarland turbidity standard. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C with 65% relative humidity. Carriers were used within 2 hours of drying. For each lot of test substance, test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 24.40°C with 41.27% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. Each test vessel was shaken thoroughly and vortex mixed. The entire volume of the subculture broths were individually transferred to the surface of a filter membrane (0.2 µm porosity), pre-wetted with 10.0 mL of sterile saline and filtered using a vacuum pump. Each filter membrane was washed with ≥50 mL of sterile saline and was then aseptically placed on the surface of a Chocolate Agar plate. All plates were incubated for 2 days at 35-37°C in CO_2. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: No protocol deviation or amendments were required for this study.

14. MRID 492486-14, "AOAC Germicidal Spray Method," Test Organism: *Klebsiella pneumoniae* (ATCC 4352). For product Brace. Study conducted at ATS Labs by Jill Ruhme. Study completion date – October 30, 2013. Project Number A15093.

Thw study was conducted against *Klebsiella pneumoniae* (ATCC 4352). Testing was conducted using one batch of test substance Brace, Batch 2028-030. Testing was performed according to ATS Laboratory Protocol No. SRC62040513.GS.15 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loopful of

stock slant culture was transferred to an initial 10 mL tube of Nutrient Broth growth medium, mixed and incubated for 24±2 hours at 35-37°C. A 10 µL aliquot of this culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C and was mixed thoroughly prior to use. The test culture was vortexed and allowed to stand for ≥10 minutes prior to removing the upper portion leaving behind any clumps or debris and pooling the cultures in a sterile vessel for use in testing. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C with 40% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 23.8°C with 40.3% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessels were shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: No protocol deviations or amendments were required for this study.

15. **MRID 492486-15, "AOAC Germicidal Spray Method," Test Organism: *Klebsiella pneumonia* – NDM-1 positive (CDC 1000527). For product Brace. Study conducted at ATS Labs by Jill Ruhme. Study completion date – October 25, 2013. Project Number A15094.**

The study was conducted against *Klebsiella pneumonia*, NDM-1 positive (CDC 1000527). Testing was conducted using one batch of test substance Brace, Batch 2028-030. Testing was performed according to ATS Laboratory Protocol No. SRC62040513.GS.16 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of Nutrient Broth growth medium, mixed and incubated for 24±2 hours at 35-37°C. A 10 µL aliquot of this culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C and was mixed thoroughly prior to use. The test culture was vortexed and allowed to stand for ≥10 minutes prior to removing the upper portion leaving behind any clumps or debris and pooling the cultures in a sterile vessel for use in testing. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C with 50% relative humidity. The carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal

position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 24.8°C with 38.8% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessels were shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Antibiotic sensitivity testing was performed using a representative culture from the day of testing to verify the stated antibiotic resistance pattern. This testing was performed at the University of Minnesota Physicians Outreach Laboratory in Minneapolis, Minnesota and confirmed antibiotic resistance. This testing was not performed under EPA Good Laboratory Practices (40 CFR Part 160).

Note: No protocol deviations or amendments were required for this study.

16. MRID 492486-16, "AOAC Germicidal Spray Method," Test Organism: *Klebsiella pneumonia* Carbapenem Resistant (ATCC BAA-1705). For product Brace. Study conducted at ATS Labs by Gracia Schroeder. Study completion date – October 22, 2013. Project Number A15095.

The study was conducted against *Klebsiella pneumonia* Carbapenem Resistant (ATCC BAA-1705). Testing was conducted using one batch of test substance Brace, Batch 2028-030. Testing was performed according to ATS Laboratory Protocol No. SRC62040513.GS.17 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of Nutrient Broth growth medium, mixed and incubated for 24±2 hours at 35-37°C. A 10 µL aliquot of this culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C and was mixed thoroughly prior to use. The test culture was vortexed and allowed to stand for ≥10 minutes prior to removing the upper portion leaving behind any clumps or debris and pooling the cultures in a sterile vessel for use in testing. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C with 41% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 24.6°C with 39.5% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessels were shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Antibiotic susceptibility testing of *Klebsiella pneumoniae* Carbapenem Resistant (ATCC BAA-1705) was performed by ATS Labs to verify the antimicrobial resistance pattern. A modified Hodge test was performed on a representative culture from the day of testing to confirm that the test organism is antibiotic resistant. In addition, antibiotic sensitivity testing was verified on a representative culture at the University of Minnesota Physicians Outreach Laboratory in Minneapolis, Minnesota. This testing was not performed under EPA Good Laboratory Practices (40 CFR Part 160).

Note: No protocol deviations occurred during this study.

17. MRID 492486-17, "AOAC Germicidal Spray Method," Test Organism: Extended-Spectrum Beta-lactamase (ESBL) producing *Klebsiella pneumonia* (ATCC 700603). For product Brace. Study conducted at ATS Labs by Gracia Schroeder. Study completion date – October 28, 2013. Project Number A15062.

The study was conducted against Extended-Spectrum Beta-lactamase (ESBL) producing *Klebsiella pneumonia* (ATCC 700603). Testing was conducted using two batches of test substance Brace, Batch 2028-030 and 2028-031. Testing was performed according to ATS Laboratory Protocol No. SRC62040513.GS.18 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of Nutrient Broth growth medium, mixed and incubated for 24±2 hours at 35-37°C. A 10 µL aliquot of this culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C and was mixed thoroughly prior to use. The test culture was vortexed and allowed to stand for ≥10 minutes prior to removing the upper portion leaving behind any clumps or debris and pooling the cultures in a sterile vessel for use in testing. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 31 minutes at 35-37°C with 52.3% relative humidity. The carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 23.3°C with 32.2% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessels were shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Antibiotic resistance testing of Extended-Spectrum Beta-lactamase (ESBL) producing *Klebsiella pneumonia* (ATCC 700603) was performed by ATS Labs to verify the antimicrobial resistance pattern of an ESBL. The Etest® assay was performed on a representative culture from the day of testing to confirm that the test organism is antibiotic resistant. In addition, antibiotic sensitivity testing was verified on a

representative culture at the University of Minnesota Physicians Outreach Laboratory in Minneapolis, Minnesota. University of Minnesota performed. This testing was not performed under EPA Good Laboratory Practices (40 CFR Part 160).

Note: No protocol deviations occurred during this study.

18. **MRID 492486-18, "AOAC Germicidal Spray Method," Test Organism: *Listeria monocytogenes* (ATCC 19117). For product Brace. Study conducted at ATS Labs by Gracia Schroeder. Study completion date – October 29, 2013. Project Number A15096.**

The study was conducted against *Listeria monocytogenes* (ATCC 19117). Testing was conducted using one batch of test substance Brace, Batch 2028-030. Testing was performed according to ATS Laboratory Protocol No. SRC62040513.GS.19 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of Brain Heart Infusion broth, mixed and incubated for 24±2 hours at 35-37°C. A 10 µL aliquot of this culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C and was mixed thoroughly prior to use. The test culture was vortexed and allowed to stand for ≥10 minutes prior to removing the upper portion leaving behind any clumps or debris and pooling the cultures in a sterile vessel for use in testing. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C with 42% relative humidity. The carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 23.9°C with 41.7% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Brain Heart Infusion + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessels were shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: No protocol deviations occurred during this study.

19. **MRID 492486-19, "AOAC Germicidal Spray Method," Test Organism: *Neisseria elongata* (ATCC 25295). For product Brace. Study conducted at ATS Labs by Gracia Schroeder. Study completion date – October 22, 2013. Project Number A15146.**

The study was conducted against *Neisseria elongata* (ATCC 25295). Testing was conducted using one batch of test substance Brace, Batch 2028-031. Testing was performed according to ATS Laboratory Protocol No. SRC62040513.GS.20 (copy provided). The product was received as ready to use (RTU) aerosol spray. A culture of

the test organism was prepared by using a stock plate to inoculate multiple agar plates and incubating for 4 days at 35-37°C in CO_2. Following incubation, an organism suspension was prepared in Fluid Thioglycolate Medium to target $1x10^8$ CFU/mL. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 25-30°C with 65% relative humidity. The carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 23.3°C with 43% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Brain Heart Infusion Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The entire volume of the subculture broths were individually transferred to the surface of a filter membrane (0.2 µm porosity). Each filter membrane was removed aseptically from the filter unit and placed on the surface of a Chocolate Agar plate for recovery of the test organism. All subcultures were incubated for 2 days at 35-37° in CO_2. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: No protocol deviations occurred during this study.

20. MRID 492486-20, "AOAC Germicidal Spray Method," Test Organism: *Proteus mirabilis* (ATCC 25933). For product Brace. Study conducted at ATS Labs by Anne Stemper. Study completion date – October 11, 2013. Project Number A15155.

The study was conducted against *Proteus mirabilis* (ATCC 25933). Testing was conducted using one batch of test substance Brace, Batch 2028-031. Testing was performed according to ATS Laboratory Protocol No. SRC62040513.GS.21 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of Nutrient Broth growth medium, mixed and incubated for 24±2 hours at 35-37°C. A 10 µL aliquot of this culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C and was mixed thoroughly prior to use. The test culture was vortexed and allowed to stand for ≥10 minutes prior to removing the upper portion leaving behind any clumps or debris and pooling the cultures in a sterile vessel for use in testing. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C with 50% relative humidity. The carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed

to remain wet for 2 minutes at 23.3°C with 38.7% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessels were shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: No protocol deviations occurred during this study.

21. MRID 492486-21, "AOAC Germicidal Spray Method," Test Organism: *Proteus vulgaris* (ATCC 9920). For product Brace. Study conducted at ATS Labs by Jill Ruhme. Study completion date – September 26, 2013. Project Number A15147.

The study was conducted against *Proteus vulgaris* (ATCC 9920). Testing was conducted using one batch of test substance Brace, Batch 2028-031. Testing was performed according to ATS Laboratory Protocol No. SRC62040513.GS.22 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loopful of stock slant culture was transferred to tryptic Soy agar plates with 5% sheep blood and incubated for 4 days at 35-37°C. Following incubation, the organism was suspended in sterile diluent and a 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 25-30°C with 65% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 23.2°C with 39.1% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessels were shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: No protocol deviations occurred during this study.

22. MRID 492486-22, "AOAC Germicidal Spray Method," Test Organism: *Pseudomonas putida* (ATCC 12633). For product Brace. Study conducted at ATS Labs by Joshua Luedtke. Study completion date – October 11, 2013. Project Number A15148.

The study was conducted against *Pseudomonas putida* (ATCC 12633). Testing was conducted using one batch of test substance Brace, Batch 2028-031. Testing was performed according to ATS Laboratory Protocol No. SRC62040513.GS.24 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loopful of

stock slant culture was transferred to tryptic soy agar plates with 5% sheep blood and incubated for 4 days at 35-37°C. Following incubation, the organism was suspended in sterile diluent and a 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 25-30°C with 65% relative humidity. The carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 23.0°C with 38.6% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessels were shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: No protocol deviations occurred during this study.

23. **MRID 492486-23, "AOAC Germicidal Spray Method," Test Organism: *Salmonella enterica* serovar enteritidis (ATCC 13076). For product Brace. Study conducted at ATS Labs by Anne Stemper. Study completion date – Oct 17, 2013. Project Number A15133.**

The study was conducted against *Salmonella enterica* serovar enteritidis (ATCC 13076). Testing was conducted using two batches of test substance Brace, Batch 2028-031 and Batch 1971-095. Testing was performed according to ATS Laboratory Protocol No. SRC62040513.GS.25 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of Nutrient Broth growth medium, mixed and incubated for 24±2 hours at 35-37°C. A 10 µL aliquot of this culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). For testing performed on July 23, 2013, one additional daily transfer was prepared. The final test culture was incubated for 48-54 hours at 35-37°C and was mixed thoroughly prior to use. The test culture was vortexed and allowed to stand for ≥10 minutes prior to removing the upper portion leaving behind any clumps or debris and pooling the cultures in a sterile vessel for use in testing. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C with 50-51.2% relative humidity. The carriers were used within 2 hours of drying. For testing performed on June 17, 2014, the test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. For testing performed on July 23, 2014, the test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 3 seconds. For testing

performed on June 17, 2014, the carriers were allowed to remain wet for 2 minutes at 23.9°C with 39.57% relative humidity. For testing performed on July 23, 2014, the carriers were allowed to remain wet for 2 minutes at 23.9°C with 38.6% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessels were shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Per the sponsors request, the efficacy test was repeated with Formula e0029-002H (Batch 1971-095) with 60 carriers because there was an insufficient amount of Formula #1178-172 (Batch 2028-031) remaining to complete the repeat test after initially producing failing data due to positive carriers confirmed contamination. Results of testing with Formula #1178-172 (Batch 2028-031) and Formula e0029-002H (Batch 1971-095) are presented in the study report.

24. MRID 492486-24, "AOAC Germicidal Spray Method," Test Organism: *Salmonella enterica* serovar Paratyphi B (ATCC 8759). For product Brace. Study conducted at ATS Labs by Jill Ruhme. Study completion date – October 17, 2013. Project Number A15424.

The study was conducted against *Salmonella enterica* serovar Paratyphi B (ATCC 8759). Testing was conducted using two batches of test substance Brace, Batch 2028-030 and 2028-031. Testing was performed according to ATS Laboratory Protocol No. SRC62080213.GS.2 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of Nutrient Broth growth medium, mixed and incubated for 24±2 hours at 35-37°C. A 10 µL aliquot of this culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C and was mixed thoroughly prior to use. The test culture was vortexed and allowed to stand for ≥10 minutes prior to removing the upper portion leaving behind any clumps or debris and pooling the cultures in a sterile vessel for use in testing. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C with 50% relative humidity. The carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 3 seconds. The carriers were allowed to remain wet for 2 minutes at 24.2°C with 48.3% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: No protocol deviations occurred during this study.

25. MRID 492486-25, "AOAC Germicidal Spray Method," Test Organism: *Salmonella enterica* serovar Typhi (ATCC 6539). For product Brace. Study conducted at ATS Labs by Jill Ruhme. Study completion date – October 17, 2013. Project Number A15144.

The study was conducted against *Salmonella enterica* serovar Typhi (ATCC 6539). Testing was conducted using one batch of test substance Brace, Batch 2028-031. Testing was performed according to ATS Laboratory Protocol No. SRC62040513.GS.27 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of Nutrient Broth growth medium, mixed and incubated for 24±2 hours at 35-37°C. A 10 µL aliquot of this culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C and was mixed thoroughly prior to use. The test culture was vortexed and allowed to stand for ≥10 minutes prior to removing the upper portion leaving behind any clumps or debris and pooling the cultures in a sterile vessel for use in testing. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C with 50% relative humidity. The carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 23.5°C with 39.0% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: No protocol deviations occurred during this study.

26. MRID 492486-26, "AOAC Germicidal Spray Method," Test Organism: *Serratia marcescens* (ATCC 14756). For product Brace. Study conducted at ATS Labs by Anne Stemper. Study completion date – October 16, 2013. Project Number A15134.

The study was conducted against *Serratia marcescens* (ATCC 14756). Testing was conducted using one batch of test substance Brace, Batch 2028-031. Testing was performed according to ATS Laboratory Protocol No. SRC62040513.GS.28 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of Tryptic Soy Broth growth medium, mixed and incubated for 24±2 hours at 35-37°C. A 10 µL aliquot of this culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). One additional daily transfer was prepared. The final test

culture was incubated for 48-54 hours at 35-37°C. The test culture was vortexed and allowed to stand for ≥10 minutes prior to removing the upper portion leaving behind any clumps or debris and pooling the cultures in a sterile vessel for use in testing. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C with 50% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 23.4°C with 37.9% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. All subcultures were incubated for 48±2 hours at 35-37°C. The vessel was shaken thoroughly. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: No protocol deviations or amendments were required for this study.

27. MRID 492486-27, "AOAC Germicidal Spray Method," Test Organism: *Shigella dysenteriae* (ATCC 11835). For product Brace. Study conducted at ATS Labs by Gracia Schroeder. Study completion date – October 21, 2013. Project Number A15135.

The study was conducted against *Shigella dysenteriae* (ATCC 11835). Testing was conducted using one batch of test substance Brace, Batch 2028-031. Testing was performed according to ATS Laboratory Protocol No. SRC62040513.GS.29 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of Nutrient Broth growth medium, mixed and incubated for 24±2 hours at 35-37°C. A 10 µL aliquot of this culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C. The test culture was vortexed and allowed to stand for ≥10 minutes prior to removing the upper portion leaving behind any clumps or debris and pooling the cultures in a sterile vessel for use in testing. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 25-30°C with 65% relative humidity. The carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 23.82°C with 38.80% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.07% Lecithin +

0.5% Tween 80 to neutralize. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: No protocol deviations or amendments were required for this study.

28. MRID 492486-28, "AOAC Germicidal Spray Method," Test Organism: Methicillin Resistant *Staphylococcus aureus* - MRSA (ATCC 33592). For product Brace. Study conducted at ATS Labs by Gracia Schroeder. Study completion date – October 15, 2013. Project Number A15136.

The study was conducted against Methicillin Resistant *Staphylococcus aureus* - MRSA (ATCC 33592). Testing was conducted using one batch of test substance Brace, Batch 2028-031. Testing was performed according to ATS Laboratory Protocol No. SRC62040513.GS.30 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of Synthetic Broth growth medium, mixed and incubated for 24±2 hours at 35-37°C. A 10 µL aliquot of this culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C and was mixed thoroughly prior to use. The test culture was vortexed and allowed to stand for ≥10 minutes prior to removing the upper portion leaving behind any clumps or debris and pooling the cultures in a sterile vessel for use in testing. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C with 50% relative humidity. The carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 23.75°C with 39.47% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Antimicrobial susceptibility testing was performed by ATS Labs for Methicillin Resistant *Staphylococcus aureus* - MRSA (ATCC 33592) to verify the MRSA antibiotic resistance pattern stated. The Kirby Bauer susceptibility assay was performed by ATS Labs utilizing a representative culture from the day of testing confirming antibiotic resistance of the MRSA test organism. In addition, antibiotic sensitivity testing was performed at the University of Minnesota Physicians Outreach Laboratory. This testing was not performed under EPA Good Laboratory Practices (40 CFR Part 160).

Note: No protocol deviations or amendments were required for this study.

29. MRID 492486-29, "AOAC Germicidal Spray Method," Test Organism: Community Acquired Methicillin Resistant *Staphylococcus aureus* – CA-MRSA Genotype USA 300 (NARSA NRS 384). For product Brace. Study conducted at ATS Labs by Gracia Schroeder. Study completion date – October 15, 2013. Project Number A15129.

The study was conducted against Community Acquired Methicillin Resistant *Staphylococcus aureus* – CA-MRSA Genotype USA 300 (NARSA NRS 384). Testing was conducted using two batches of test substance Brace, Batch 2028-030 and Batch 2028-031. Testing was performed according to ATS Laboratory Protocol No. SRC62060513.GS.1 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of Synthetic Broth growth medium, mixed and incubated for 24±2 hours at 35-37°C. A 10 µL aliquot of this culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C and was mixed thoroughly prior to use. The test culture was vortexed and allowed to stand for ≥10 minutes prior to removing the upper portion leaving behind any clumps or debris and pooling the cultures in a sterile vessel for use in testing. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C with 49% relative humidity. The carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 24.8°C with 38.3% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Antimicrobial susceptibility testing was performed by ATS Labs for Community Acquired Methicillin Resistant *Staphylococcus aureus* – CA-MRSA Genotype USA 300 (NARSA NRS 384) to verify the MRSA antibiotic resistance pattern stated. The Kirby Bauer susceptibility assay was performed utilizing a representative culture from the day of testing confirming antibiotic resistance of the MRSA test organism. In addition, antibiotic sensitivity testing was performed at the University of Minnesota Physicians Outreach Laboratory. This testing was not performed under EPA Good Laboratory Practices (40 CFR Part 160).

Note: No protocol deviations or amendments were required for this study.

30. MRID 492486-30, "AOAC Germicidal Spray Method," Test Organism: Community Acquired Methicillin Resistant *Staphylococcus aureus* – CA-MRSA Genotype USA 400 (NARSA NRS 123). For product Brace. Study

conducted at ATS Labs by Gracia Schroeder. Study completion date – October 15, 2013. Project Number A15130.

The study was conducted against Community Acquired Methicillin Resistant *Staphylococcus aureus* – CA-MRSA Genotype USA 400 (NARSA NRS 123). Testing was conducted using two batches of test substance Brace, Batch 2028-030 and Batch 2028-031. Testing was performed according to ATS Laboratory Protocol No. SRC62060513.GS.2 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of Synthetic Broth growth medium, mixed and incubated for 24±2 hours at 35-37°C. A 10 µL aliquot of this culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C and was mixed thoroughly prior to use. The test culture was vortexed and allowed to stand for ≥10 minutes prior to removing the upper portion leaving behind any clumps or debris and pooling the cultures in a sterile vessel for use in testing. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C with 41% relative humidity. The carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 24.6°C with 39.5% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Antimicrobial susceptibility testing was performed by ATS Labs for Community Acquired Methicillin Resistant *Staphylococcus aureus* – CA-MRSA Genotype USA 400 (NARSA NRS 123) to verify the MRSA antibiotic resistance pattern stated. The Kirby Bauer susceptibility assay was performed utilizing a representative culture from the day of testing confirming antibiotic resistance of the MRSA test organism. In addition, antibiotic sensitivity testing was performed at the University of Minnesota Physicians Outreach Laboratory. This testing was not performed under EPA Good Laboratory Practices (40 CFR Part 160).

Note: No protocol deviations or amendments were required for this study.

31. MRID 492486-31, "AOAC Germicidal Spray Method," Test Organism: *Staphylococcus epidermidis* (ATCC 12228). For product Formula Brace. Study conducted at ATS Labs by Anne Stemper. Study completion date – October 16, 2013. Project Number A15137.

The study was conducted against *Staphylococcus epidermidis* (ATCC 12228). Two batches of product Brace were tested, Batch 2028-031 and Batch 1971-095.

Testing was performed in accordance with ATS Laboratory Protocol No. SRC62040513.GS.31 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of Synthetic Broth growth medium, mixed and incubated for 24±2 hours at 35-37°C. A 10 µL aliquot of this culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). For testing of performed on August 6, 2013, one additional daily transfer was prepared. The final test culture was incubated for 48-54 hours at 35-37°C and was mixed thoroughly prior to use. The test culture was vortexed and allowed to stand for ≥10 minutes prior to removing the upper portion leaving behind any clumps or debris and pooling the cultures in a sterile vessel for use in testing. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C with 50% relative humidity. The carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds for testing performed on June 17, 2013 and for 3 seconds for testing performed on August 6, 2013. The carriers were allowed to remain wet for 2 minutes at 23.8°C with 39.0% relative humidity for testing performed on June 17, 2013 and at 24.9°C with 36.8% relative humidity for testing performed on August 6, 2013. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Subcultures showing growth were subcultured, stained and/or biochemically assayed to confirm or rule out the presence of the test organism. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Per the sponsor's request, the efficacy test was repeated for Batch 1971-095 with 60 carriers because the efficacy test with Batch 2028-031 failed due to an insufficient amount of Batch 2028-031 remaining to complete the repeat test. Results of testing with Batch 2028-031 and Batch 1971-095 are presented in the study report.

32. MRID 492486-32, "AOAC Germicidal Spray Method," Test Organism: *Streptococcus pneumoniae* – Penicillin Resistant and Multidrug Resistant (ATCC 700677). For product Brace. Study conducted at ATS Labs by Gracia Schroeder. Study completion date – October 22, 2013. Project Number A15063.

The study was conducted against *Streptococcus pneumoniae* – Penicillin Resistant and Multidrug Resistant (ATCC 700677). Testing was conducted using two batches of test substance Brace, Batch 2028-030 and Batch 2028-031. Batch 2028-030 was tested on 6/11/2013 and Batch 2028-031 was tested on 6/25/2013. Testing was performed in accordance with ATS Laboratory Protocol No. SRC62040513.GS.34 (copy provided). The product was received as ready to use (RTU) aerosol spray. From the stock plate, multiple Tryptic Soy agar plates with 5% sheep blood (BAP) were inoculated with the test organism. The plates were incubated for 2-4 days at 35-37°C in CO_2.

Following incubation, the organism was suspended in Fluid Thioglycollate Medium to target 1 x 10^8 CFU/mL. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30-33 minutes at 25-30°C with 65% relative humidity. The carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 23.70°C and 23.8°C with 40.2% - 40.68% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Brain Heart Infusion Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C in CO_2. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Antibiotic susceptibility testing of *Streptococcus pneumoniae* – Penicillin Resistant and Multidrug Resistant (ATCC 700677) was performed by ATS Labs to verify the antimicrobial resistance pattern stated. The Etest® assay was performed on a representative culture from the day of testing to confirm that the test organism is Penicillin and Multidrug resistant. In addition, antibiotic sensitivity testing was performed at the University of Minnesota Physicians Outreach Laboratory. This testing was not performed under EPA Good Laboratory Practices (40 CFR Part 160).

33. MRID 492486-33, "AOAC Germicidal Spray Method," Test Organism: *Streptococcus pyogenes*, (ATCC 12384). For product Brace. Study conducted at ATS Labs by Joshua Luedtke. Study completion date – October 11, 2013. Project Number A15138.

The study was conducted against *Streptococcus pyogenes*, (ATCC 12384). Testing was conducted using one batch of test substance Brace, Batch 2028-031. Testing was performed in accordance with ATS Laboratory Protocol No. SRC62040513.GS.32 (copy provided). The product was received as ready to use (RTU) aerosol spray. From the stock plate, multiple Tryptic Soy agar plates with 5% sheep blood (BAP) were inoculated with the test organism. The plates were incubated for 4 days at 35-37°C in 6.0% CO_2. Following incubation, the organism was suspended in Fluid Thioglycollate Medium to target 1 x 10^8 CFU/mL. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 25-30°C with 65% relative humidity. The carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 22.74°C with 44.76%

relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps to 20 mL of Brain Heart Infusion Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C in 6.0% CO_2. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: No protocol deviation or amendments were required for this study.

34. MRID 492486-34, "AOAC Germicidal Spray Method," Test Organism: *Streptococcus salivarius*, (ATCC 7073). For product Brace. Study conducted at ATS Labs by Joshua Luedtke. Study completion date – October 11, 2013. Project Number A15139.

The study was conducted against *Streptococcus salivarius*, (ATCC 7073). Testing was conducted using one batch of test substance Brace, Batch 2028-031. Testing was performed in accordance with ATS Laboratory Protocol No. SRC62040513.GS.33 (copy provided). The product was received as ready to use (RTU) aerosol spray. From the stock plate, multiple Tryptic Soy agar plates with 5% sheep blood (BAP) were inoculated with the test organism. The plates were incubated for 4 days at 35-37°C in 6.0% CO_2. Following incubation, the organism was suspended in Fluid Thioglycollate Medium to target 1 x 10^8 CFU/mL. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 25-30°C with 65% relative humidity. The carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 23.9°C with 39.0% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps to 20 mL of Brain Heart Infusion Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C in 6.0% CO_2. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: No protocol deviation or amendments were required for this study.

35. MRID 492486-35 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Adenovirus type 14," for product Brace, by Shanen Conway. Study conducted at ATS Labs. Study completion date – October 28, 2013. Project Number A15483.

The study was conducted against de Wit strain of Adenovirus type 14 (ATCC VR-15). Two lots of test substance Brace, Batch 2028-030 and Batch 2028-031, were tested using ATS Laboratory Protocol No. SRC62081913.ADV (copy provided). On the day of

testing, one aliquot of stock virus (ATS Labs Lot A14-1) was thawed and maintained at refrigerated temperature until used in the assay. The fetal bovine serum was added to the stock virus culture to yield a 5% organic soil load. Human lung carcinoma cells A-549 (ATCC CCL-185) were used as the host cell line. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM), supplemented with 5% heat inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. Films of virus were prepared at staggered intervals by spreading 200 µL of virus uniformly over the bottoms of three 100 x 15 mm sterile glass Petri dishes. The virus films were dried at 20.0°C for 20 minutes at 40% relative humidity. The dried virus film was thoroughly sprayed for 3 seconds until wet at a distance of 6-8 inches and held covered for 3 minutes at 20.0°C. The dried films were scraped with a cell scraper and the virus-test substance mixtures were immediately passed through prepared individual Sephadex columns utilizing the syringe plungers to detoxify the mixtures. The filtrates (10^{-1} dilution) were then titered by 10-fold serial dilution and were assayed for infectivity. The A-549 cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions from the test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus film recovery, cytotoxicity, neutralization and cell viability.

Note: No protocol deviations or amendments were reported for this study.

36. MRID 492486-36 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Echovirus type 12," for product Brace, by Shanen Conway. Study conducted at ATS Labs. Study completion date – September 10, 2013. Project Number A15160.

The study was conducted against Travis 2-85 strain of Echovirus type 12 (ATCC VR-1563). One lot of test substance Brace, Batch 2028-029, was tested using ATS Laboratory Protocol No. SRC62032013.ECHV (copy provided). On the day of testing, one aliquot of stock virus (ATS Labs Lot E12-3) was thawed and maintained at refrigerated temperature until used in the assay. The fetal bovine serum was added to the stock virus culture to yield a 5% organic soil load. Rhesus Monkey Kidney cells (RMK) (obtained from Diagnostic Hybrids, Athens, OH) were used as the host cell line. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM), supplemented with 2% heat inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. Films of virus were prepared at staggered intervals by spreading 200 µL of virus uniformly over the bottoms of two 100 x 15 mm sterile glass Petri dishes. The films were dried at 20.0°C for 20 minutes at 40% relative humidity. The dried virus film was thoroughly sprayed for 2 seconds until wet at a distance of 6-8 inches and held covered for 1 minute at 21.0°C. The dried films were scraped with a cell scraper and the virus-test substance mixtures were immediately passed through prepared individual Sephadex columns utilizing the syringe plungers to detoxify the mixtures. The filtrates (10^{-1} dilution) were then titered by 10-fold serial dilution and were assayed for infectivity. The RMK cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions from the test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus film recovery, cytotoxicity, neutralization and cell viability.

Note: No protocol deviations or amendments were reported for this study.

37. MRID 492486-37 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Bovine Viral Diarrhea Virus as a Surrogate Virus for Human Hepatitis C Virus," for product Brace, by Shanen Conway. Study conducted at ATS Labs. Study completion date – September 13, 2013. Project Number A15119.

The study was conducted against Bovine Viral Diarrhea Virus as a Surrogate Virus for Human Hepatitis C Virus. The test virus was Oregon C24v-genotype 1 strain of Bovine Viral Diarrhea virus (BVDV) originally obtained from the National Veterinary Services Laboratories (NVSL), Ames, IA. Two lots of test substance Brace, Batch 2028-030 and Batch 2028-029, were tested using ATS Laboratory Protocol No. SRC62032013.BVD (copy provided). Bovine turbinate (BT) cells (ATCC CRL-1390) were used as the host cell line. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM) supplemented with 5% (v/v) non-heat inactivated horse serum. The medium was also supplemented with the following: 10 µg/ml gentamicin, 100 units/mL penicillin, and 2.5 µg /ml amphotericin B. On the day of testing, one aliquot of stock virus (ATS Labs Lot BVDF-20) was thawed and maintained at refrigerated temperature until used in the assay. The stock virus culture was adjusted to contain 5% horse serum to yield a 5% organic soil load. Films of virus were prepared at staggered intervals by spreading 200 µL of virus uniformly over the bottoms of 100 x 15 mm sterile glass Petri dishes. The virus films were dried at 20.0°C for 20 minutes at 40% relative humidity. The dried virus films were sprayed for 2 seconds at a distance of 6-8 inches and held covered for 30 seconds at 21.0°C. The dried films were scraped with a cell scraper and the virus-test substance mixtures were immediately passed through prepared individual Sephadex columns utilizing the syringe plungers to detoxify the mixtures. The filtrates (10^{-1} dilution) were then titered by 10-fold serial dilution and were assayed for infectivity. The BT cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions from the test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus film recovery, cytotoxicity, neutralization and cell viability.

Note: No protocol deviations or amendments were reported for this study.

38. MRID 492486-38 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Poliovirus type 1," for product Brace, by Shanen Conway. Study conducted at ATS Labs. Study completion date – August 5, 2013. Project Number A15168.

The study was conducted against Chat strain of Poliovirus type 1 (ATCC VR-1562). One lot of test substance Brace, Batch 2028-029, was tested using ATS Laboratory Protocol No. SRC62052113.POL (copy provided). Vero cells (ATCC CCL-81) were used as the host cell line. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM) supplemented with 5% (v/v) heat-inactivated FBS and the following: 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg /mL amphotericin B. On the day of testing, one aliquot of stock virus (ATS Labs Lot PC2-17) was thawed and maintained at refrigerated temperature until used in the assay. The stock virus culture was adjusted to contain 5% fetal bovine serum to yield a 5% organic

soil load. Films of virus were prepared at staggered intervals by spreading 200 µL of virus uniformly over the bottoms of 100 x 15 mm sterile glass Petri dishes. The virus film was dried at 15.5°C for 20 minutes at 55% relative humidity. The dried virus film was thoroughly sprayed for 2 seconds until wet at a distance of 6-8 inches and held covered for 1 minute at 21.0°C. The dried films were scraped with a cell scraper and the virus-test substance mixtures were immediately passed through prepared individual Sephadex columns utilizing the syringe plungers to detoxify the mixtures. The filtrates (10^{-1} dilution) were then titered by 10-fold serial dilution and were assayed for infectivity. The Vero cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions from the test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus film recovery, cytotoxicity, neutralization and cell viability.

Note: No protocol deviations or amendments were reported for this study.

39. MRID 492486-39 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces" Virus: Newcastle disease virus for product Brace. Study Director Mary J. Miller. Study conducted at ATS Labs. Study completion date – September 16, 2013. Project Number A15120.

The study was conducted against the B1, Hitchner or Blacksburg strain of Newcastle disease virus (ATCC VR-108). Testing was performed on two lots of product Brace, Batch 2028-030 and Batch 2028-029, using ATS Labs Protocol No. SRC62032013.NEW (copy provided). The stock virus was prepared by collecting the allantoic fluid from inoculated nine day old fertilized embryonated chicken eggs. The cells were disrupted and cell debris removed by centrifugation at approximately 2000 RPM for five minutes at approximately 4°C. The supernatant was removed, aliquoted, and the high titer stock virus was stored at -70°C until the day of use. On the day of use, an aliquot of stock virus (ATS Labs Lot NDV-43B) was removed, thawed and maintained at a refrigerated temperature until used in the assay. The stock virus culture was adjusted with fetal bovine serum to yield a 5% organic soil load. Chicken embryo fibroblast (CEF) cells (obtained from Charles River) were used as the host cell line. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM), supplemented with 2% heat activated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL amphotericin B, 5% (v/v) tryptose phosphate broth and 2.0 mM L-glutamine. Films of the virus were prepared by spreading 0.2 mL inoculum uniformly over the bottoms of 100 x 15 mm sterile glass Petri dishes. The virus films were dried at 20°C in a relative humidity of 40% for 20 minutes. The dried virus film was sprayed for 2 seconds with the test substance and held covered for 30 seconds at 21.0°C. Following the exposure time, the plates were individually scraped with a cell scraper to resuspend the contents and the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates (10^{1} dilution) were then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity. Four assay wells (containing CEF cells) were inoculated in quadruplicate with 0.1 mL of the dilutions prepared and were incubated at 36-38°C in a humidified atmosphere at 5-7% CO_2. The cultures were scored periodically for seven days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus film recovery, virus stock titer confirmation, cytotoxicity, neutralization and cell viability.

Note: No protocol deviations or amendments were reported for this study.

40. MRID 492486-40 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces" Virus: Influenza B Virus (ATCC VR-823, Strain B/Hong Kong/5/72) for product Brace. Study Director Mary J. Miller. Study conducted at ATS Labs. Study completion date – September 16, 2013. Project Number A15132.

The study was conducted against Influenza B Virus H1N1 (ATCC VR-823, Strain B/Hong Kong/5/72). Testing was performed on two lots of product Brace, Batch 2028-030 and Batch 2028-031, using ATS Labs Protocol No. SRC62060513.FLUB (copy provided). The B/Hong Kong/5/72 strain of Influenza B virus was obtained from the American Type Culture Collection, Manassas, VA (ATCC VR-823). The stock virus was prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells were disrupted and cell debris removed by centrifugation at approximately 2000 RPM for five minutes at approximately 4°C. The supernatant was removed, aliquoted, and the high titer stock virus was stored at -70°C until the day of use. On the day of use, an aliquot of stock virus (ATS Labs Lot NFB-5) was removed, thawed and maintained at a refrigerated temperature until used in the assay. The stock virus culture was adjusted with fetal bovine serum to yield a 5% organic soil load. Rhesus Monkey Kidney cells (RMK) (obtained from Diagnostic Hybrids, Athens, OH) were used as the host cell line. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM), supplemented with 1% heat activated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. Films of Influenza B virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of 100 x 15 mm sterile glass Petri dishes. The virus films were dried at 20°C in a relative humidity of 40% for 20 minutes. The dried virus film was sprayed with the test substance for 2 seconds and held covered for 30 seconds at 21.0°C. Following the exposure time, the plates were individually scraped with a cell scraper to resuspend the contents and the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates (10^1 dilution) were then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity. Four assay wells (containing RMK cells) were inoculated in quadruplicate with 0.1 mL of the dilutions prepared and were incubated at 36-38°C in a humidified atmosphere at 5-7% CO_2. The cultures were scored periodically for seven days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus film recovery, virus stock titer confirmation, cytotoxicity, neutralization and cell viability.

Note: No protocol deviations or amendments were reported for this study.

41. MRID 492486-41 "Virucidal Hard-Surface Efficacy Test: SARS Associated Coronavirus," for product Brace, by S. Steve Zhou. Study conducted at Microbiotest Labs. Study completion date – December 4, 2013. Project Number 121-202.

The study was conducted against the SARS-Associated Coronavirus (CDC Strain 200300592; Zeptometrix). Two lots of test substance Brace, Batch 1836-132 and Batch 1836-133, were tested using Microbiotest Laboratory Protocol No. 121.1c.06.05.13 (copy provided). On the day of testing, one aliquot of stock virus in RPMI 1640 10% fetal bovine serum was thawed and maintained at refrigerated

temperature until used in the assay. The stock virus culture was adjusted with RPMI 1640 to yield a 5% organic soil load. Vero E6 cells (ATCC CRL-1586) were used as the host cell line. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM), supplemented with 10% heat inactivated fetal bovine serum. Films of virus were prepared at staggered intervals by spreading 400 µL of virus uniformly over 4 in^2 area of 10 cm diameter sterile glass Petri dishes. The virus films were dried at 20.0°C for 30 minutes at 44.6-44.9% relative humidity. The dried virus film was sprayed with the test substance for 2 seconds at a distance of 6-8 inches and held covered at 20-21.0°C for 30 seconds at 44.6-44.9% relative humidity. The dried films were neutralized with 2 mL of MEM + 20% FBS + 0.1% Polysorbate 80 + 1% Lecithin + 5% HEPES and scraped with a cell scraper. A 0.5 mL of neutralized virus-test substance mixture was serially diluted in MEM + 2% FBS and assayed for infectivity. The Vero E6 cells in multiwell culture dishes were inoculated in quadruplicate with 1.0 mL of the dilutions from the test and control groups and were allowed absorption for 21.2 hours at 36±2°C in a humidified atmosphere of 5±1% CO_2. After absorption, the cultures were refed and incubated for an additional six (6) days at 36±2°C in a humidified atmosphere of 5±1% CO_2 for a total of a 7 days incubation period. The host cells were examined microscopically for the absence or presence of infectious virions, CPE, and cytotoxicity. Controls included those for dried virus film recovery, cytotoxicity, neutralization and cell viability.

Note: The protocol deviations or amendments were reported for this study were found to be acceptable.

42. MRID 492486-42 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Virus: Human Coronavirus for product Brace, by Shanen Conway. Study conducted at ATS Labs. Study completion date – August 5, 2013. Project Number A15182.

The study was conducted against the 229E strain of Human Coronavirus (ATCC VR-740). Two lots of test substance Brace, Batch 2028-030 and Batch 2028-031, were tested using ATS Laboratory Protocol No. SRC62061113.COR (copy provided). On the day of testing, one aliquot of stock virus (ATS Labs Lot HCV-66) was thawed and maintained at refrigerated temperature until used in the assay. The stock virus culture was adjusted with fetal bovine serum to yield a 5% organic soil load. WI-38 (human lung) cells (ATCC CCL-75) were used as the host cell line. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM), supplemented with 2% heat inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. Films of virus were prepared at staggered intervals by spreading 200 µL of virus uniformly over the bottoms of 100 x 15 mm sterile glass Petri dishes. The virus films were dried at 20.0°C for 20 minutes at 50% relative humidity. For each lot of test substance, one dried virus film was thoroughly sprayed with test substance for 2 seconds at a distance of 6-8 inches and held covered for 30 seconds 20.0°C. Near the end of the exposure time, the dried films were scraped with a cell scraper and at the end of the exposure time the virus-test substance mixtures were immediately passed through prepared individual Sephadex columns utilizing the syringe plungers to detoxify the mixtures. The filtrates (10^{-1} dilution) were then titered by 10-fold serial dilution and were assayed for infectivity. The WI-38 cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions from the test and control groups and were incubated at 31-35°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 11 days for the absence or presence of CPE, cytotoxicity, and for

viability. Controls included those for dried virus film recovery, cytotoxicity, neutralization and cell viability.

Note: No protocol deviations or amendments were reported for this study.

43. MRID 492486-43, "Fungicidal Germicidal Spray Method," Test Organism: *Trichophyton mentagrophytes*, (ATCC 9533). For product Brace. Study conducted at ATS Labs by Anne Stemper. Study completion date – October 16, 2013. Project Number A15097.

The study was conducted against *Trichophyton mentagrophytes*, (ATCC 9533). Testing was conducted using one batch of test substance Brace, Batch 2028-031. Testing was performed using ATS Laboratory Protocol No. SRC62051513.FGS (copy provided). The product was received as ready to use (RTU) aerosol spray. From a stock culture of the test organism, 30 Sabouraud Dextrose Agar plates were inoculated and incubated at 25-30°C for 10 days. The mycelia were removed using a sterile device and a conidia suspension was prepared. The conidia suspension was passed through sterile gauze to remove hyphal fragments and a ten-fold dilution of the filtered conidial suspension was performed using sterile saline. The conidial count estimated using a hemacytometer was $7.5x10^7$ conidia/mL. The test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C with 40% relative humidity. The carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 23.36°C with 42.31% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Sabouraud Dextrose Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessel was shaken thoroughly. All neutralized subcultures were incubated for 10 days at 25-30°C. The Potato Dextrose agar plate subcultures were incubated for 44-76 hours at 25-30°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Protocol amendments reported in this study were reviewed and found to be acceptable. No protocol deviations were required for this study.

44. MRID 492486-44, "Fungicidal Germicidal Spray Method," Test Organism: *Aspergillus niger* (ATCC 6275). For product Brace. Study conducted at ATS Labs by Anne Stemper. Study completion date – October 16, 2013. Project Number A15118.

The study was conducted against *Aspergillus niger*, (ATCC 6275). Testing was conducted using two batches of test substance Brace, Batch 2028-030 and Batch 2028-031. Testing was performed using ATS Laboratory Protocol No. SRC62040513.FGS.1 (copy provided). The product was received as ready to use (RTU) aerosol spray. From a

stock culture of the test organism, a flask of Sabouraud Agar (modified) was inoculated and incubated at 25-30°C for 7 days. The mycelia/conidia were removed from the agar and a conidia suspension was prepared. The conidia suspension was passed through sterile gauze to remove hyphal fragments and a ten-fold dilution of the filtered conidial suspension was performed using sterile saline. The conidial count estimated using a hemacytometer was $9.5x10^7$ conidia/mL. The test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C with 40% relative humidity. The carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 3 minutes at 22.9°C with 42.5% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Sabouraud Dextrose Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessel was shaken thoroughly. All neutralized subcultures were incubated for 10 days at 25-30°C. The Sabouraud Dextrose agar plate subcultures were incubated for 44-76 hours at 25-30°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: No protocol amendments or deviations were required for this study.

45. MRID 492486-45, "Fungicidal Germicidal Spray Method," Test Organism: *Candida albicans*, (ATCC 10231). For product Brace. Study conducted at ATS Labs by Joshua Luedtke. Study completion date – October 28, 2013. Project Number A15102.

The study was conducted against *Candida albicans*, (ATCC 10231). Testing was conducted using one batch of test substance Brace, Batch 2028-031. This was tested using ATS Laboratory Protocol No. SRC62040513.FGS.2 (copy provided). The product was received as ready to use (RTU) aerosol spray. From a stock culture of the test organism, a sufficient number of Sabouraud Dextrose Agar plates were inoculated and incubated at 25-30°C for 3 days. Following incubation, the organism was suspended in Butterfield's Buffer to target 1 x 10^8 CFU/mL. The test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 25-30°C with 65% relative humidity. The carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at 22.44°C with 40.79% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to

20 mL of Sabouraud Dextrose Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessel was shaken thoroughly. All neutralized subcultures were incubated for 2 days at 25-30°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: No protocol amendments or deviations were required for this study.

46. MRID 492486-46, "Fungicidal Germicidal Spray Method," Test Organism: *Penicillium chrysogenum* (ATCC 10109). For product Brace. Study conducted at ATS Labs by Anne Stemper. Study completion date – October 11, 2013. Project Number A15149.

The study was conducted against *Penicillium chrysogenum* (ATCC 10109). Testing was conducted using two batches of test substance Brace, Batch 2028-030 and Batch 2028-031. Testing was performed using ATS Laboratory Protocol No. SRC62040513.FGS.3 (copy provided). The product was received as ready to use (RTU) aerosol spray. From a stock culture of the test organism, 20 Sabouraud Dextrose Agar plates were inoculated and incubated at 25-30°C for 15 days. The mycelia were removed using a sterile device and a conidia suspension was prepared. The conidia suspension was passed through sterile gauze to remove hyphal fragments and a ten-fold serial dilution of the prepared suspension was performed. The conidial count estimated using a hemacytometer was 3.40×10^8 conidia/mL. The test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (18 mm x 36 mm) each in a Petri dish matted with two pieces of filter were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 25-30°C with 66% relative humidity. The carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 3 minutes at 22°C with 52% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Sabouraud Dextrose Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. The vessel was shaken thoroughly. All neutralized subcultures were incubated for 10 days at 25-30°C. The Sabouraud Dextrose agar plate subcultures were incubated for 44-76 hours at 25-30°. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: No protocol amendments or deviations were required for this study.

V. RESULTS:

MRID Number	Organism	No. Exhibiting Growth/ Total No. Carriers Tested			Carrier Population (Log_{10} CFU/ Carrier)
		Batch 2028-029	Batch 2028-030	Batch 2028-031	
2 Minutes Exposure Time					
492486-01	*Pseudomonas aeruginosa* (ATCC 15442)	1/60	0/60	1/60	5.33
	Salmonella enterica (ATCC 10708)	0/60	0/60	0/60	5.36
	Staphylococcus aureus (ATCC 6538)	0/60	0/60	0/60	6.29
492486-02	*Acinetobacter baumannii* (MDR) (ATCC 19606)		0/10	0/10	5.54
492486-03	*Acinetobacter calcoaceticus* (ATCC 17902)		0/10	0/10	4.89
492486-04	*Bordetella pertussis* (ATCC 12743)		0/10	0/10	7.15
492486-05	*Burkholderia cepacia* (ATCC 25416)		0/10		4.84
492486-06	*Corynebacterium diphtheriae* (ATCC 11913)		0/10		4.52
492486-07	*Enterobacter aerogenes* MDR (ATCC 29751)		0/10		6.32
492486-08	*Enterococcus faecalis* (ATCC 828)		0/10		5.08
492486-09	*Enterococcus faecalis* VRE (ATCC 51575)		0/10		5.25
492486-10	*Enterococcus faecium* (MDR) (ATCC 51559)		0/10	0/10	5.9
492486-11	*Escherichia coli* 0157:H7 (ATCC 43888)		0/10		4.88
492486-12	*Escherichia coli* (ESBL) (ATCC BAA-196)		0/10		5.29
492486-13	*Haemophilus influenzae* (ATCC 33930)		0/10	0/10	5.16
492486-14	*Klebsiella pneumoniae* (ATCC 4352)		0/10		4.86
492486-15	*Klebsiella pneumoniae* NDM-1 (CDC 1000527)		0/10		5.19
492486-16	*Klebsiella pneumoniae* (CPR) (ATCC BAA-1705)		0/10		5.71
492486-17	*Klebsiella pneumonia* ESBL (ATCC 700603)		0/10	0/10	5.05

492486-18	*Listeria monocytogenes* (ATCC 19117)		0/10		5.47
492486-19	*Neisseria elongata* (ATCC 25295)		Test Date: 6/18/13	3/10	5.12
			Test Date: 7/09/13	0/10	5.74
492486-20	*Proteus mirabilis* (ATCC 25933)			0/10	6.51
492486-21	*Proteus vulgaris* (ATCC 9920)			0/10	4.96
492486-22	*Pseudomonas putida* (ATCC 12633)			0/10	4.29
492486-23	*Salmonella enterica* serovar enteritidis (ATCC 13076)		Test Date: 6/17/13	1/10	5.51
		Batch 1971-095 (Test Date: 7/23/13); 0/60			4.98
492486-24	*Salmonella enterica* serovar Paratyphi B (ATCC 8759)		0/10	0/10	5.41
492486-25	*Salmonella enterica* serovar Typhi (ATCC 6539)			0/10	4.67
492486-26	*Serratia marcescens* (ATCC 14756)			0/10	6.41
492486-27	*Shigella dysenteriae* (ATCC 11835)			0/10	4.74
492486-28	Methicillin Resistant *Staphylococcus aureus* - MRSA (ATCC 33592)			0/10	6.74
492486-29	*Staphylococcus aureus* – CA-MRSA Genotype USA 300 (NARSA NRS 384)		0/10	0/10	6.45
492486-30	*Staphylococcus aureus* – CA-MRSA Genotype USA 400 (NARSA NRS 123)		0/10	0/10	6.10
492486-31	*Staphylococcus epidermidis* (ATCC 12228)		Test Date:6/17/14	1/10	5.04
492486-31	*Staphylococcus epidermidis* (ATCC 12228)	Batch 1971-095 (Test Date:8/6/14); 0/60			5.01
492486-32	*Streptococcus pneumoniae* Penicillin Resistant and Multidrug Resistant (ATCC 700677)	Test Date 6/11/13			4.73
			0/10		
		Test Date 6/25/13			5.08
				0/10	
492486-33	*Streptococcus pyogenes* (ATCC 12384)			0/10	5.67

492486-34	*Streptococcus salivarius* (ATCC 7073)			0/10	6.24

MRID Number	Organism	Results			Dried Virus Count
3 Minutes Exposure Time					
492486-35	Adenovirus type 14 (ATCC VR-15)		**Lot No. 2028-030**	**Lot No. 2028-031**	
		10^{-1} to 10^{-8} Dilutions	Complete inactivation	Complete inactivation	$10^{5.75}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	

MRID Number	Organism	Results		Dried Virus Count
1 Minute Exposure Time				
492486-36	Echovirus type 12 (ATCC VR-1563)		**Lot No. 2028-029**	
		10^{-1} to 10^{-9} Dilutions	Complete inactivation	$10^{5.00}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.50}$	
492486-38	Poliovirus type 1 (ATCC VR-1562)	10^{-1} to 10^{-8} Dilutions	Complete inactivation	$10^{5.75}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.50}$	

MRID Number	Organism	Results			Dried Virus Count
30 Seconds Exposure Time					
492486-37	Bovine Viral Diarrhea Virus (strain Oregon C24v-genotype 1)		**Lot No. 2028-030**	**Lot No. 2028-029**	
		10^{-1} to 10^{-4} Dilutions	Complete inactivation	Complete inactivation	$10^{4.50}$ $TCID_{50}$/ 0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
492486-39	Newcastle Disease Virus (ATCC VR-108)	10^{-1} to 10^{-7} Dilutions	Complete inactivation	Complete inactivation	$10^{4.75}$ $TCID_{50}$/ 0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
492486-40	Influenza B Virus (ATCC VR-823, Strain B/Hong Kong/5/72)		**Lot No. 2028-030**	**Lot No. 2028-031**	$10^{4.50}$ $TCID_{50}$/0.1 mL
		10^{-1} to 10^{-7} Dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/0.1 mL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
492486-42	Human Coronavirus (ATCC VR-740)	10^{-1} to 10^{-8} Dilutions	Complete inactivation	Complete inactivation	$10^{6.00}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
492486-41	SARS-Associated Coronavirus,	**@30 seconds contact time**	**Lot No. 1836-132**	**Lot No. 1836-133**	$10^{6.85}$ $TCID_{50}$/0.4

MRID Number	Organism	Results			Dried Virus Count
	(CDC Strain 200300592; Zeptometrix)	10^{-2} to 10^{-7} Dilutions	Cytotoxicity was seen at 10^{-2} dilution	Cytotoxicity was seen at 10^{-2} dilution	mL
		$TCID_{50}$/0.4 mL	$\leq 10^{2.10}$	$\leq 10^{2.10}$	

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested			Carrier Population Average (Log_{10} CFU/carrier)
		Batch 2028-029	Batch 2028-030	Batch 2028-031	
2 Minutes Exposure Time					
492486-43	*Trichophyton mentagrophytes* (ATCC 9533)			0/10	4.96
492486-45	*Candida albicans* (ATCC 10231)			0/10	5.66
3 Minutes Exposure Time					
492486-44	*Aspergillus niger* (ATCC 6275)		0/10	0/10	5.40
492486-46	*Penicillium chrysogenum* (ATCC 10109)		0/10	0/10	6.35

VI. CONCLUSIONS

1.) The submitted efficacy data **does support** the use of the ready-to-use spray Brace as a disinfectant against the following bacteria on hard, non-porous surfaces with a 5% organic soil load for a 2 minute contact time:

Pseudomonas aeruginosa (ATCC 15442)	MRID 492486-01
Salmonella enterica (ATCC 10708)	MRID 492486-01
Staphylococcus aureus (ATCC 6538)	MRID 492486-01

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

2.) The submitted efficacy data **does support** the use of the ready-to-use spray Brace as a disinfectant against the following bacteria on hard, non-porous surfaces with a 5% organic soil load for a 2 minute contact time:

Acinetobacter baumannii MDR (ATCC 19606)	MRID 492486-02
Acinetobacter calcoaceticus (ATCC 17902)	MRID 492486-03
Bordetella pertussis (ATCC 12743)	MRID 492486-04
Burkholderia capacia (ATCC 25416)	MRID 492486-05

Corynebactium diptheriae (ATCC 11913)	MRID 492486-06
Enterobacter aerogenes MDR (ATCC 29751)	MRID 492486-07
Enterococcus faecalis (ATCC 828)	MRID 492486-08
Enterococcus faecalis - Vancomycin Resistant (VRE) (ATCC 51575)	MRID 492486-09
Enterococcus faecium VRE + MDR (ATCC 51559)	MRID 492486-10
Escherichia coli O157:H7 (ATCC 43888)	MRID 492486-11
Escherichia coli ESBL (ATCC BAA 196)	MRID 492486-12
Haemophilus influenzae (ATCC 33930)	MRID 492486-13
Klebsiella pneumoniae (ATCC 4352)	MRID 492486-14
Klebsiella pneumoniae NDM-1 positive (CDC 1000527)	MRID 492486-15
Klebsiella pneumoniae Carbapenem resistant (ATCC BAA 1705)	MRID 492486-16
Klebsiella pneumoniae ESBL (ATCC 700603)	MRID 492486-17
Listeria monocytogenes (ATCC 19117)	MRID 492486-18
Proteus mirabilis (ATCC 25933)	MRID 492486-20
Proteus vulgaris (ATCC 9920)	MRID 492486-21
Pseudomonas putida (ATCC 12633)	MRID 492486-22
Salmonella enterica serovar paratyphi B (ATCC 8759)	MRID 492486-24
Salmonella enterica serovar typhi (ATCC 6539)	MRID 492486-25
Serratia marcescens (ATCC 14756)	MRID 492486-26
Shigella dysenteriae (ATCC 11835)	MRID 492486-27
Staphylococcus aureus - Methicillin resistant (HA-MRSA) (ATCC 33592)	MRID 492486-28
Staphylococcus aureus - CA-MRSA USA-300 (NARSA NRS 384)	MRID 492486-29
Staphylococcus aureus - CA-MRSA USA-400 (NARSA NRS 123)	MRID 492486-30
Streptococcus pneumoniae PRSP (ATCC 700677)	MRID 492486-32
Streptococcus pyogenes (ATCC 12384)	MRID 492486-33
Streptococcus salivarius (ATCC 7073)	MRID 492486-34

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth. Antibiotic resistance was verified in antibiotic-resistant test strains.

3.) The submitted efficacy data **does not support** the use of the ready-to-use spray Brace as a disinfectant against the following bacteria on hard, non-porous surfaces with a 5% organic soil load for a 2 minute contact time:

Neisseria elongata (ATCC 25295)	MRID 492486-19

The studies submitted did not demonstrate acceptable killing in the subcultures of the required number of carriers tested. Testing demonstrated failing data for 3 out of 10 carriers in which subcultures for 2 of the 3 failed carriers were confirmed positive for the test organism. With this level of failure, retesting of 10 carriers is unacceptable. Neutralization confirmation testing showed positive growth of the microorganisms.

Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

4.) The submitted efficacy data **does support** the use of the ready-to-use spray Brace as a disinfectant against the following bacteria on hard, non-porous surfaces with a 5% organic soil load for a 2 minute contact time:

Salmonella enterica serovar enteritidis (ATCC 13076)	MRID 492486-23
Staphylococcus epidermidis (ATCC 12228)	MRID 492486-31

Although initial testing submitted demonstrated failing data for 1 out of 10 carriers tested, complete killing was observed in the subcultures of the retested full set of sixty (60) carriers. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

5.) The submitted efficacy data **does support** the use of the ready-to-use spray Brace as a disinfectant against the following virus on hard, non-porous surfaces with a 5% organic soil load for a 3-minute contact time:

Adenovirus 14 (ATCC VR-15)	MRID 492486-35

Complete inactivation (no growth) was indicated in all dilutions tested. Recoverable virus titers of at least 10^4 were achieved. Neutralization control demonstrated growth.

6.) The submitted efficacy data **does support** the use of the ready-to-use spray Brace as a disinfectant against the following viruses on hard, non-porous surfaces with a 5% organic soil load for a 1-minute contact time:

Echovirus Type 12 (ATCC VR – 1563)	MRID 492486-36
Poliovirus Type 1 (ATCC VR – 1662)	MRID 492486-38

Complete inactivation was demonstrated or at least a 3-log reduction in titer was shown beyond the cytotoxic level. Recoverable virus titers of at least 10^4 were achieved. No cytotoxicity was observed. Neutralization control demonstrated growth.

7.) The submitted efficacy data **does support** the use of the ready-to-use spray Brace as a disinfectant against the following viruses on hard, non-porous surfaces with a 5% organic soil load for a 30-second contact time:

Hepatitis C Virus (Bovine Viral Diarrhea Virus; strain Oregon C24v – genotype 1)	MRID 492486-37
Newcastle Disease virus (ATCC VR – 108)	MRID 492486-39
Influenza B Virus (Strain B/Hong Kong/5/72; ATCC VR-823)	MRID 492486-40
SARS Coronavirus (CDC 200300592, Zeptometrix)	MRID 492486-41
Human Coronavirus (ATCC VR-740, Strain 229E)	MRID 492486-42

Complete inactivation was demonstrated or at least a 3-log reduction in titer was shown beyond the cytotoxic level. Recoverable virus titers of at least 10^4 were achieved. Neutralization control demonstrated growth.

8.) The submitted efficacy data **does support** the use of the ready-to-use spray Brace as a disinfectant against the following fungi on hard, non-porous surfaces with a 5% organic soil load for a 2-minute contact time:

Trichophyton mentagrophytes (ATCC 9533)	MRID 492486-43
Candida albicans (ATCC 10231)	MRID 492486-45

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

9.) The submitted efficacy data **does support** the use of the ready-to-use spray Brace as a disinfectant against the following fungi on hard, non-porous surfaces with a 5% organic soil load for a 3-minute contact time:

Aspergillus niger (ATCC 6275)	MRID 492486-44
Penicillin chrysogenum (ATCC 10109)	MRID 492486-46

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

VII. RECOMMENDATIONS

1. The label claims that ready-to-use spray Brace is a disinfectant against the following bacteria on hard, non-porous surfaces for a 2 minute contact time:

   Pseudomonas aeruginosa (ATCC 15442)
   Salmonella enterica (ATCC 10708)
   Staphylococcus aureus (ATCC 6538)

   These claims are acceptable as they are supported by the submitted data

2. The label claims that ready-to-use spray Brace is a disinfectant against the following bacteria on hard, non-porous surfaces for a 2 minute contact time:

   Acinetobacter baumannii MDR (ATCC 19606)
   Acinetobacter calcoaceticus (ATCC 17902)
   Bordetella pertussis (ATCC 12743)
   Burkholderia capacia (ATCC 25416)
   Corynebactium diptheriae (ATCC 11913)
   Enterobacter aerogenes MDR (ATCC 29751)

Enterococcus faecalis (ATCC 828)
Enterococcus faecalis - Vancomycin Resistant (VRE) (ATCC 51575)
Enterococcus faecium VRE + MDR (ATCC 51559)
Escherichia coli O157:H7 (ATCC 43888)
Escherichia coli ESBL (ATCC BAA 196)
Haemophilus influenzae (ATCC 33930)
Klebsiella pneumoniae (ATCC 4352)
Klebsiella pneumoniae NDM-1 positive (CDC 1000527)
Klebsiella pneumoniae Carbapenem resistant (ATCC BAA 1705)
Klebsiella pneumoniae ESBL (ATCC 700603)
Listeria monocytogenes (ATCC 19117)
Proteus mirabilis (ATCC 25933)
Proteus vulgaris (ATCC 9920)
Pseudomonas putida (ATCC 12633)
Salmonella enterica serovar paratyphi B (ATCC 8759)
Salmonella enterica serovar typhi (ATCC 6539)
Serratia marcescens (ATCC 14756)
Shigella dysenteriae (ATCC 11835)
Staphylococcus aureus - Methicillin resistant (HA-MRSA) (ATCC 33592)
Staphylococcus aureus - CA-MRSA USA-300 (NARSA NRS 384)
Staphylococcus aureus - CA-MRSA USA-400 (NARSA NRS 123)
Streptococcus pneumoniae PRSP (ATCC 700677)
Streptococcus pyogenes (ATCC 12384)
Streptococcus salivarius (ATCC 7073)
Salmonella enterica serovar enteritidis (ATCC 13076)
Staphylococcus epidermidis (ATCC 12228)

These claims are acceptable as they are supported by the submitted data.

3. The label claims that ready-to-use spray Brace is a disinfectant against the following bacteria on hard, non-porous surfaces for a 2 minute contact time:

   Neisseria elongata (ATCC 25295)

These claims are <u>unacceptable</u> as they are <u>not supported</u> by the submitted data.

4. The label claims that the ready-to-use spray Brace is a disinfectant against the following virus on hard, non-porous surfaces for a 3-minute contact time:

   Adenovirus 14 (ATCC VR – 15)

   These claims are acceptable as they are supported by the submitted data.

5. The label claims that the ready-to-use spray Brace is a disinfectant against the following viruses on hard, non-porous surfaces with a 5% organic soil load for a 1-minute contact time:

   Echovirus Type 12
   Poliovirus Type 1

   These claims are acceptable as they are supported by the submitted data.

6. The label claims that the ready-to-use spray Brace is a disinfectant against the following viruses on hard, non-porous surfaces for a 30-second contact time:

   Hepatitis C Virus (Bovine Viral Diarrhea Virus; strain Oregon C24v – genotype 1)
   Newcastle Disease virus (ATCC VR – 108)
   Influenza B Virus (Strain B/Hong Kong/5/72; ATCC VR-823)
   SARS Coronavirus (CDC 200300592, Zeptometrix)
   Human Coronavirus (ATCC VR-740, Strain 229E)

   These claims are acceptable as they are supported by the submitted data.

7. The label claims that the ready-to-use spray Brace is a disinfectant against the following fungi on hard, non-porous surfaces for a 2-minute contact time:

   Trichophyton mentagrophytes (ATCC 9533)
   Candida albicans (ATCC 10231)

   These claims are acceptable as they are supported by the submitted data.

7. The label claims that the ready-to-use spray Brace is a disinfectant against the following fungi on hard, non-porous surfaces for a 3-minute contact time:

   Aspergillus niger (ATCC 6275)
   Penicillin chrysogenum (ATCC 10109)

   These claims are acceptable as they are supported by the submitted data.

LABEL RECOMMENDATIONS

- Remove disinfectant on hard, non-porous surfaces for a 2 minute contact time claims for the following microorganisms:
  Neisseria elongata (ATCC 25295)
  Data was not submitted to support these claims
- For all directions for use for disinfection including Page 14 on amended proposed label, the label must qualify the microorganisms with the approved use contact times.
- On page 12, revise "Newcastle Disease" to read "Newcastle Disease Virus." Also, correct/add the appropriate AATC numbers for Newcastle Disease Virus and Influenza A (H1N1).